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COLCHICINE, CYTOCHALASIN B, AND PIGMENT MOVEMENTS IN OVARIAN AND INTEGUMENTARY ERYTHROPHORES OF THE PRAWN, *PALAEMONETES VULGARIS*[1]

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The erythrophores of the prawn, *Palaemonetes vulgaris,* are controlled by pigment-concentrating and pigment-dispersing hormones (Brown, Webb, and Sandeen, 1952). These hormones appear to exert their primary actions at the plasma membrane, not entering the cells to initiate pigment migration (Fingerman and Connell, 1968; Fingerman, 1969).

In several vertebrates and the fiddler crab, *Uca pugilator,* drug studies have implicated both microtubules (Wright, 1955; Malawista, 1965, 1971a; Wikswo and Novales, 1969; Schliwa and Bereiter-Hahn, 1973; Lambert and Crowe, 1973) and microfilaments (Malawista, 1971b; McGuire and Moellmann, 1972; Lyerla and Novales, 1972; Lambert and Crowe, 1973; Robison and Charlton, 1973) as cell structures possibly involved in pigment granule movement within chromatophores. Colchicine and cytochalasin B have been favorite tools for investigators studying microtubules and microfilaments because colchicine can dissociate microtubules (Borisy and Taylor, 1967) and cytochalasin B has as one effect the disruption of microfilaments (Schroeder, 1970; Wessells, Spooner, Ash, Bradley, Luduena, Taylor, Wrenn, and Yamada, 1971).

Practically all the investigators who have studied the effect of colchicine on chromatophoric pigment migration have observed that this drug inhibits pigment aggregation (*i.e.,* concentration). The notable exception as far as the experiments to be described below are concerned is the report by Robison and Charlton (1973) who stated that they observed no inhibition of pigment aggregation in the dark erythrophores on the surface of the ovary of the prawn, *Palaemonetes vulgaris,* after these cells were treated with colchicine. However, at the time Robison and Charlton published their data, we had already performed some unpublished experiments with integumentary erythrophores of this prawn and found that colchicine did indeed inhibit pigment concentration in these chromatophores. A series of experiments was then devised to reconcile the difference between the observations of Robison and Charlton and our unpublished observations. In addition, the effects of cytochalasin B and colchicine on pigment dispersion in this prawn were determined. Robison and Charlton reported that cytochalasin B inhibited pigment aggregation in *Palaemonetes,* but neither they nor anyone else has previously tested the effect of either colchicine or cytochalasin B on pigment dispersion in the erythrophores of this prawn. Aside from the report of Robison and Charlton, there is no other publication dealing with the effects of these drugs on chromatophores of *Palaemonetes vulgaris.*

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Materials and Methods

The specimens of the prawn, *Palaemonetes vulgaris,* used in the experiments described below, were collected in the vicinity of Woods Hole, Massachusetts, by members of the Supply Department of the Marine Biological Laboratory. The Hogben and Slome (1931) scheme was used to stage the erythrophores. According to the Hogben and Slome system, stage 1 represents maximal pigment concentration, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. Prawns with maximally concentrated red pigment were obtained by placing intact individuals in white containers for at least one hour, whereas prawns with maximally dispersed red pigment were obtained by putting individuals into black containers for a similar period of time.

The erythrophores used in these experiments were observed *in vitro.* In some experiments the erythrophores on the surface of the ovaries were used, in others we used the erythrophores of the epidermis attached to the portion of the exoskeleton dorsal to the heart. The ovarian erythrophores were obtained by carefully removing the ovaries from the prawns. To obtain the integumentary chromatophores, a portion of the carapace approximately 3 mm long and 4 mm wide was removed and then cut into two pieces, each about 3×2 mm, one serving as the experimental piece, the other as the control. These portions of isolated carapace consisted of exoskeleton and chromatophore-containing epidermis. The pieces of carapace and ovaries were placed in Pantin's crustacean saline (Pantin, 1934) that was diluted until it was isosmotic with the blood of the prawn (Fingerman and Connell, 1968).

Colchicine (Sigma) was prepared in isosmotic saline. Cytochalasin B (Aldrich) was first dissolved in dimethyl sulfoxide (DMSO) and this solution was then diluted with isosmotic saline to a cytochalasin B concentration of 10 μg/ml in a 0.1% DMSO solution.

Red pigment-concentrating hormone was obtained by preparing extracts of the tritocerebral commissure in a concentration of 0.1 tritocerebral commissure equivalent per 0.025 ml. This commissure contains red pigment-concentrating hormone, but no red pigment-dispersing hormone (Brown, Webb, and Sandeen, 1952). Red pigment-dispersing hormone without red pigment-concentrating hormone was obtained by subjecting abdominal nerve cords to gel chromatography in essentially the manner described by Fingerman and Bartell (1973). The abdominal nerve cord has the highest ratio of red pigment-dispersing hormone to the antagonistic concentrating hormone (Brown, Webb, and Sandeen, 1952). By means of gel chromatography these hormones can be separated from each other (Fingerman and Bartell, 1973). Twenty-five abdominal nerve cords were extracted in 0.3 ml distilled water, applied to an 0.8×31.0 cm Bio-Gel P-6 column, and eluted with distilled water. One ml fractions were collected and made isosmotic with the prawn's blood by the addition of 0.18 ml of 400% Pantin's saline. The fractions were tested for red pigment-dispersing activity and the three having maximal activity were pooled for use in these experiments. Appropriate assays also revealed that these three fractions lacked pigment-concentrating activity.

The ovaries and pieces of integument were placed in the depressions on white porcelain spot plates. Each depression contained 0.05 ml of saline or other appropriate solution, depending upon the experiment, and one ovary or one

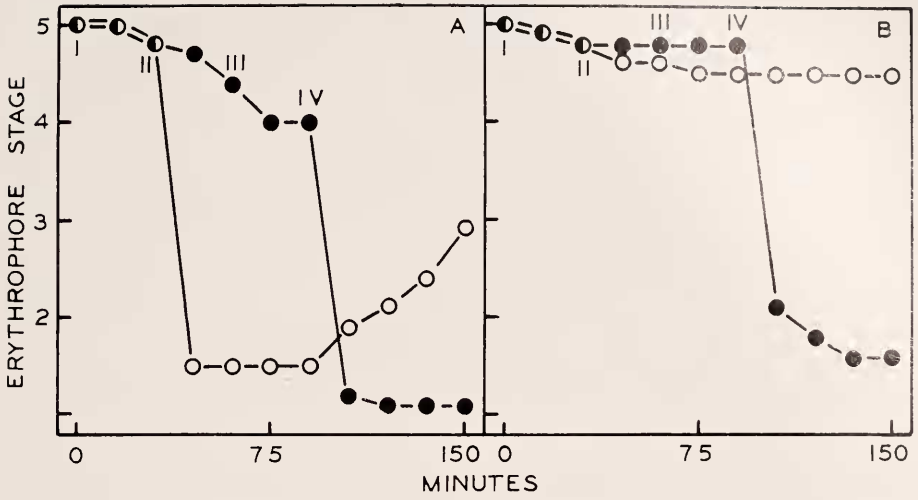

FIGURE 1. Relationships between integumentary erythrophore stage and time in minutes. A: circles represent erythrophores in saline at outset; dots, erythrophores in colchicine (25 mM) at outset. The pigment in these erythrophores was initially maximally dispersed. At the outset (at I) the erythrophores were placed in either colchicine or saline. Red pigment-concentrating hormone (RPCH) was administered to both the saline-exposed and colchicine-treated erythrophores after 30 minutes (at II). The colchicine and RPCH were replaced by saline 60 minutes after 0 time (at III) and RPCH was administered again 90 minutes after 0 time (at IV) to the erythrophores that had been in the colchicine. B: all of the erythrophores (circles and dots) were exposed to colchicine (25 mM) at the outset (at I). All received RPCH after 30 minutes (at II). Half of the erythrophore-containing pieces of carapace (dots) were placed in saline 60 minutes after 0 time (at III), and 90 minutes after 0 time (at IV) RPCH was again administered to the erythrophores that had been but were no longer in colchicine.

piece of integument. When, according to the protocol, a hormone or other solution was added to a depression containing an ovary or piece of integument and 0.05 ml of saline or other solution, the additional volume was always 0.025 ml. In every experiment either five experimental and five control ovaries or five experimental and five control pieces of integument were used. Each experiment was performed twice. Consequently, each data point in the following figures represents the mean of 10 determinations.

EXPERIMENTS AND RESULTS

Effect of 25 mM colchicine on integumentary erythrophores

The object of the first series of experiments was to determine the effects of 25 mM colchicine on pigment concentration and pigment dispersion in the integumentary erythrophores. The first experiment of this series was designed to determine, first of all, whether 25 mM colchicine inhibits pigment concentration in the integumentary erythrophores of *Palaemonetes vulgaris*. At the outset (at I in Fig. 1A) one set of pieces of exoskeleton with the adhering erythrophores was

placed in 25 mM colchicine for 30 minutes and a second set into saline. Then (at II in Fig. 1A), 0.025 ml of the tritocerebral commissure extract containing the red pigment-concentrating hormone was added to all the pieces. It is clear from Figure 1A that there was much inhibition of the pigment-concentrating response (II–III in Fig. 1A) on the part of the erythrophores exposed to the 25 mM colchicine compared with those that had been in saline alone until the hormone was added. Fifteen minutes after the hormone had been added, the pigment in the erythrophores that had been in the saline was nearly maximally concentrated whereas the erythrophores in the 25 mM colchicine were virtually unchanged after 15 minutes exposure to the hormone. Then, as part of the same experiment, we wished to see whether the effect of the colchicine was reversible. To accomplish this aim, 60 minutes from the start of the experiment (at III in Fig. 1A) the erythrophores in the colchicine were transferred to saline alone in order to wash out the drug. The saline was changed every five minutes for 30 minutes and then (at IV in Fig. 1A) the washed pieces of exoskeleton with the adhering erythrophores were placed in 0.05 ml saline to which was added 0.025 ml of the tritocerebral commissure extract and the erythrophores were observed for one more hour. The erythrophores that had been exposed to the colchicine and then washed were able to respond nearly maximally to the red pigment-concentrating hormone, thus revealing that the effect of the colchicine was reversible. The pigment in the control erythrophores (they had not received the 90-minute dose of hormone given to the washed erythrophores) began to redisperse toward the end of this experiment. Presumably, most or all of the hormone added originally had become inactivated and the red pigment began to disperse spontaneously in the absence or near absence of pigment-concentrating hormone.

An experiment was then designed to demonstrate in another fashion that 25 mM colchicine inhibits concentration of the pigment in these integumentary erythrophores. From the outset (at I in Fig. 1B) all of the pieces of exoskeleton with the adhering erythrophores were exposed to 0.05 ml of 25 mM colchicine for 30 minutes (I–II in Fig. 1B) and then 0.025 of the tritocerebral commissure extract was added to each piece (at II in Fig. 1B). It is obvious from Figure 1B (between II and III), just as from Figure 1A, that the colchicine inhibited the response to red pigment-concentrating hormone. After the erythrophores had been exposed to the hormone for 30 minutes, one-half of the pieces of exoskeleton with the adhering erythrophores was placed in saline (at III in Fig. 1B). The saline was changed every five minutes for 30 minutes in order to wash out the colchicine. After this half hour of washing, the washed pieces were placed in 0.05 ml saline to which was added 0.025 ml of the tritocerebral commissure extract (at IV in Fig. 1B). The unwashed pieces were simply placed into another 0.05 ml of 25 mM colchicine to which was added 0.025 ml of the tritocerebral commissure extract. The washed erythrophores then responded to the hormone. Again the effect of the colchicine was reversible.

The next experiment was similar to that of Figure 1A except that there were two changes in the procedure; namely, we used erythrophores that initially had maximally concentrated instead of dispersed pigment and red pigment-dispersing hormone instead of the pigment-concentrating hormone. These changes were introduced in order to determine, first of all, whether 25 mM colchicine inhibits pig-

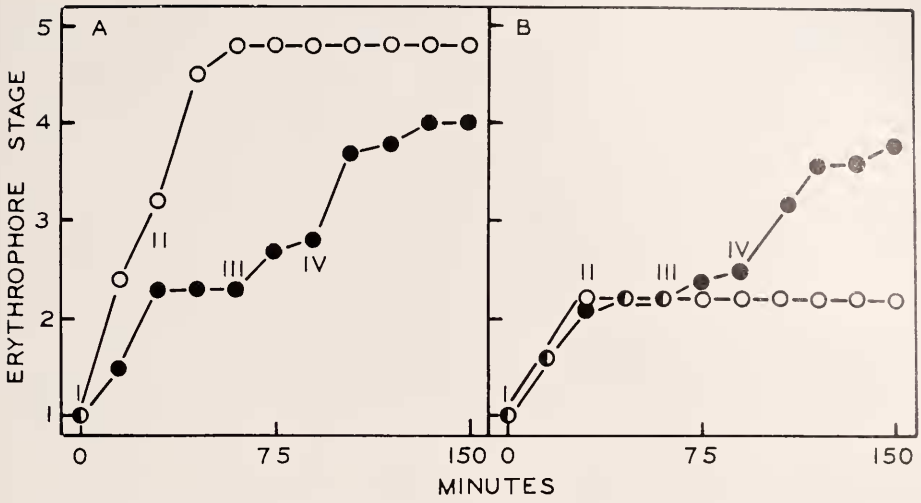

FIGURE 2. Relationships between integumentary erythrophore stage and time in minutes. A: circles represent erythrophores in saline at outset; dots, erythrophores in colchicine (25 mM) at outset. The pigment in these erythrophores was initially maximally concentrated. At the outset (at I) the erythrophores were placed in either colchicine or saline. Red pigment-dispersing hormone (RDPH) was administered to both the saline-exposed and colchicine-treated erythrophores after 30 minutes (at II). After 60 minutes from 0 time (at III) the erythrophores that had been in colchicine were washed in saline. RPDH was readministered 90 minutes after 0 time (at IV) to the erythrophores that had been in colchicine. B: all of the erythrophores (circles and dots) were exposed to colchicine (25 mM) at the outset (at I). All received RPDH after 30 minutes (at II). Half of the erythrophore-containing pieces of carapace (dots) were placed in saline 60 minutes after 0 time (at III), and 90 minutes after 0 time (at IV) RPDH was again administered to the erythrophores that had been but were no longer in colchicine.

ment dispersion in these integumentary erythrophores. One set of pieces of exoskeleton with the adhering erythrophores was placed in 25 mM colchicine and a second set in saline (at I in Fig. 2A). After 30 minutes, red pigment-dispersing hormone was administered to all of the pieces (at II in Fig. 2A) and the erythrophores were observed for 30 minutes. Then the pieces in colchicine were washed in saline (III–IV in Fig. 2A) as described above for 30 minutes and then given the hormone again (at IV in Fig. 2A). It is apparent from inspection of Figure 2A that the colchicine inhibited pigment dispersion in response to the hormone and this effect was reversible just as in the case of pigment concentration. Furthermore, the inhibitory effect of colchicine on pigment dispersion was apparent even before the hormone was administered. The spontaneous pigment dispersion that occurs when erythrophores with maximally concentrated pigment are removed from the body (I–II in Fig. 2A) was less in the erythrophores in the colchicine than in those in the saline. The dispersion that was observed in the colchicine-treated erythrophores probably occurred before sufficient colchicine had entered the cells to inhibit the pigment-dispersing mechanism.

The final experiment (Fig. 2B) of this series was the counterpart of that described for Figure 1B. In this experiment all the pieces of exoskeleton were

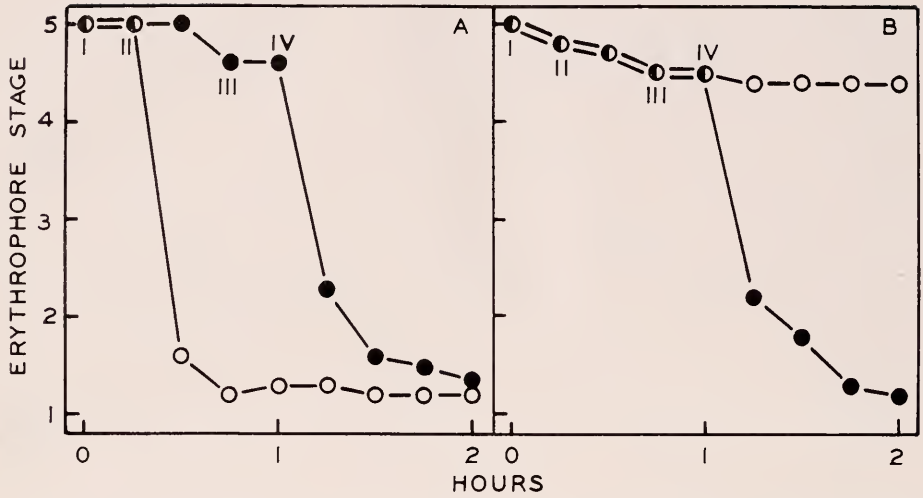

FIGURE 3. Relationships between integumentary erythrophore stage and time in hours: A: circles represent erythrophores in 0.1% dimethyl sulfoxide (DMSO) at outset; dots, erythrophores in cytochalasin B (10 μg/ml) in 0.1% DMSO at outset. The pigment in these erythrophores was initially maximally dispersed. At the outset (at I) the erythrophores were placed in either cytochalasin B or DMSO. Red pigment-concentrating hormone (RPCH) was administered to both the DMSO-exposed and cytochalasin B-treated erythrophores after 15 minutes (at II). The cytochalasin B was replaced by 0.1% DMSO 45 minutes after 0 time (at III) and RPCH was administered again 60 minutes after 0 time (at IV) to the erythrophores that had been in the cytochalasin B. B: all the erythrophores (circles and dots) were exposed to cytochalasin B (10 μg/ml) at the outset (at I). All received RPCH after 15 minutes (at II). Half of the erythrophore-containing pieces of carapace (dots) were placed in 0.1% DMSO 45 minutes after 0 time (at III), and 60 minutes after 0 time (at IV) RPCH was again administered to the erythrophores that had been but were no longer in cytochalasin B.

placed in the 25 mM colchicine solution at the outset (at I in Fig. 2B) and given the red pigment-dispersing hormone after 30 minutes (at II in Fig. 2B). Thirty minutes later half of the pieces were washed for 30 minutes in saline (III–IV in Fig. 2B) and given the hormone again (at IV in Fig. 2B). The unwashed pieces were simply placed into fresh colchicine solution to which was added the hormone (at IV in Fig. 2B). This experiment, as in Figure 2A, showed that colchicine inhibited red pigment dispersion in response to the hormone and that this inhibition was reversible.

Effect of cytochalasin B on integumentary erythrophores

The object of this series of experiments was to determine whether cytochalasin B can inhibit pigment migration in the prawn's integumentary erythrophores and if so, whether the effect is reversible. In the first experiment the effect of this drug on pigment concentration was determined. Pieces of exoskeleton with the adhering erythrophores were taken from prawns that had been kept in black containers. One set of pieces was placed into 0.05 ml of the cytochalasin B solution and the remainder served as a control, each control piece being put into 0.05 ml of 0.1%

DMSO (at I in Fig. 3A). After 15 minutes (at II in Fig. 3A) 0.025 ml of tritocerebral commissure extract was added. The tritocerebral commissure extracts used in these experiments with cytochalasin B were prepared in the same concentration ($\frac{1}{10}$ equivalent per dose) as in the colchicine experiments but in 0.1% DMSO in saline instead of in saline alone. It is clear from Figure 3A that the cytochalasin B strongly inhibited the response to the red pigment-concentrating hormone (II–III in Fig. 3A). Then, 45 minutes after the start of the experiment the pieces that had been in the cytochalasin B were placed into 0.1% DMSO and every five minutes for 15 minutes the DMSO solution was changed (III–IV in Fig. 3A). Finally, one hour after the experiment had started, the washed erythrophores were exposed to tritocerebral commissure extract again (at IV in Fig. 3A). The washed erythrophores then responded to the red pigment-concentrating hormone. The inhibition produced by cytochalasin B is clearly reversible, just as that produced by colchicine.

The next experiment (Fig. 3B) is comparable to the colchicine experiment of Figure 1B. Integumentary erythrophores from prawns in black containers were exposed to cytochalasin B for 15 minutes (I–II in Fig. 3B), pigment-concentrating hormone was added (at II in Fig. 3B), and the expected inhibition was observed (II–III in Fig. 3B). Then, 45 minutes after the experiment started (at IV in Fig. 3B), half the pieces of exoskeleton were washed for 15 minutes in 0.1% DMSO and then given the hormone again (at IV in Fig. 3B). The unwashed pieces were simply given fresh cytochalasin B plus the hormone (at IV in Fig. 3B). The washed erythrophores responded to the pigment-concentrating hormone, showing again the reversibility of the inhibition.

The final two experiments of this series (Fig. 4) utilized erythrophores that initially had maximally concentrated red pigment. In Figure 4A are shown the data obtained when one-half the pieces of exoskeleton were placed in 0.05 ml cytochalasin B and the remainder in 0.1% DMSO (at I in Fig. 4A) for 15 minutes at which time (at II in Fig. 4A) 0.025 ml of the red pigment-dispersing hormone solution was administered to each of the pieces. The response of the erythrophores in the cytochalasin B was strongly inhibited (II–III in Fig. 4A). The erythrophores in the cytochalasin B were then washed between 45 and 60 minutes from the start of the experiment in 0.1% DMSO (III–IV in Fig. 4A) and then the hormone was readministered to the washed erythrophores (at IV in Fig. 4A). The washed erythrophores then responded to the hormone. This experiment showed that the pigment-dispersing response of these erythrophores can be inhibited by cytochalasin B and that the effect is reversible. Furthermore, the inhibitory effect of the cytochalasin B on pigment dispersion was apparent even before the hormone was administered (I–II in Fig. 4A) as was also the case with the colchicine in Figure 2A.

In the next experiment all of the pieces of exoskelton with the adhering erythrophores were placed in cytochalasin B for 15 minutes (I–II in Fig. 4B) and then (at II in Fig. 4B) 0.025 ml of the red pigment-dispersing hormone were added. The pigment-dispersing response had been inhibited once again (II–III in Fig. 4B). Then, between 45 and 60 minutes from the start of the experiment (III–IV in Fig. 4B) half of the pieces were washed in 0.1% DMSO and then given another dose of the hormone (at IV in Fig. 4B). The unwashed

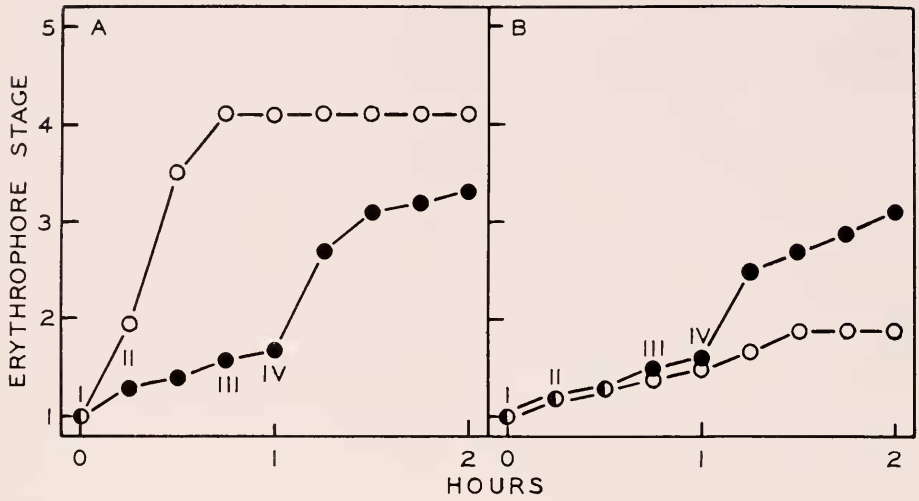

FIGURE 4. Relationships between integumentary erythrophore stage and time in hours. A: circles represent erythrophores in 0.1% dimethyl sulfoxide (DMSO) at outset; dots, erythrophores in cytochalasin B (10 μg/ml) at outset. The pigment in these erythrophores was initially maximally concentrated. At the outset (at I) the erythrophores were placed in either cytochalasin B or DMSO. Red pigment-dispersing hormone (RPDH) was administered to both the DMSO-exposed and cytochalasin B-treated erythrophores after 15 minutes (at II). The cytochalasin B was replaced by 0.1% DMSO 45 minutes after 0 time (at III) and RPDH was readministered 60 minutes after 0 time (at IV) to the erythrophores that had been in the cytochalasin B. B: all of the erythrophores (circles and dots) were exposed to cytochalasin B (10 μg/ml) at the outset (at I). All received RPDH after 15 minutes (at II). Half of the erythrophore-containing pieces of carapace (dots) were placed in 0.1% DMSO 45 minutes after 0 time (at III), and 60 minutes after 0 time (at IV) RPDH was again administered to the erythrophores that had been but were no longer in cytochalasin B.

erythrophores were simply placed in fresh cytochalasin B (at IV in Fig. 4B) to which was added 0.025 ml of the hormone solution. Only the washed erythrophores were capable at that time of dispersing their pigment.

Effect of 5 mM *colchicine on integumentary erythrophores*

The data presented in Figure 1 clearly reveal that 25 mM colchicine inhibits the response of integumentary erythrophores of the prawn, *Palaemonetes vulgaris*, to red pigment-concentrating hormone. However, Robison and Charlton (1973), as stated above, reported that colchicine does not inhibit the pigment-concentrating response in ovarian erythrophores of this prawn. Aside from the different source of the erythrophores in the prawn, integument versus ovary, there was one other very important difference between our experiments and those of Robison and Charlton. Whereas we had used a 25 mM solution of colchicine, Robison and Charlton had used only a 1 mM solution. It seemed possible that rather than there being a fundamental difference between these erythrophores from different parts of the prawn, the reason for the difference in results was due simply to the much lower concentration of colchicine that Robison and Charlton used. The following experiment was performed to examine this possibility.

Pieces of exoskeleton with the adhering erythrophores from prawns from black pans were placed into a 5 mM solution of colchicine (0.05 ml) while the control pieces were simply placed into saline. Thirty minutes later 0.025 ml of the tritocerebral commissure extract were added to each depression. The 5 mM colchicine did not inhibit the pigment-concentrating response of these erythrophores. Similarly, 5 mM colchicine did not inhibit the response of erythrophores of prawns taken from white containers to red pigment-dispersing hormone administered after the erythrophores had been incubated for 30 minutes in the drug.

Effect of colchicine on ovarian erythrophores

The final experiment was performed to confirm the supposition that the difference between our results and those of Robison and Charlton (1973) was due to the difference between the concentrations of colchicine solutions that were used and not to a fundamental difference between the pigment-concentrating mechanisms of the integumentary and ovarian erythrophores of this prawn. Four groups of ovaries were removed from prawns that had been kept in black containers so that their ovarian erythrophores would contain maximally or nearly maximally dispersed pigment. Each ovary of one group was placed into 0.05 ml 25 mM colchicine, each of the second was put into 0.05 ml of 1 mM colchicine, and each ovary of the remaining two groups was placed in 0.05 ml saline alone. Thirty minutes later, 0.025 ml of the tritocerebral commissure extract containing red pigment-concentrating hormone was added to each ovary in the colchicine solutions and to each of those in one of the two groups in saline. To each ovary in the remaining group in saline were simply added an additional 0.025 ml of saline. The erythrophores were then observed for one hour. The averaged results are presented in Figure 5 where it can be seen that the red pigment in the erythrophores in saline alone remained virtually maximally dispersed, that the pigment in the erythrophores in saline that received the tritocerebral commissure extract concentrated strongly, that the erythrophores in 25 mM colchicine showed virtually no response to the hormone, and that the erythrophores in the 1 mM colchicine responded to the red pigment-concentrating hormone but not quite as strongly as did the erythrophores in saline that received the hormone.

Discussion

It is clear from Figure 5 that the response of ovarian erythrophores to red pigment-concentrating hormone is greatly inhibited by 25 mM colchicine. The obvious explanation for the discrepancy between these data and the results of Robison and Charlton (1973) who reported that the pigment-concentrating response of ovarian erythrophores in *Palaemonetes vulgaris* is not inhibited by colchicine is that these investigators used a solution (1 mM) that was not concentrated enough to inhibit the erythrophores. Our results with integumentary erythrophores are, however, in agreement with their observation that cytochalasin B inhibits pigment concentration in ovarian erythrophores.

Both the pigment-dispersing and pigment-concentrating responses of the integumentary erythrophores of this prawn are inhibited by either colchicine or cytochalasin B. *Palaemonetes vulgaris* is the first animal for which it has been

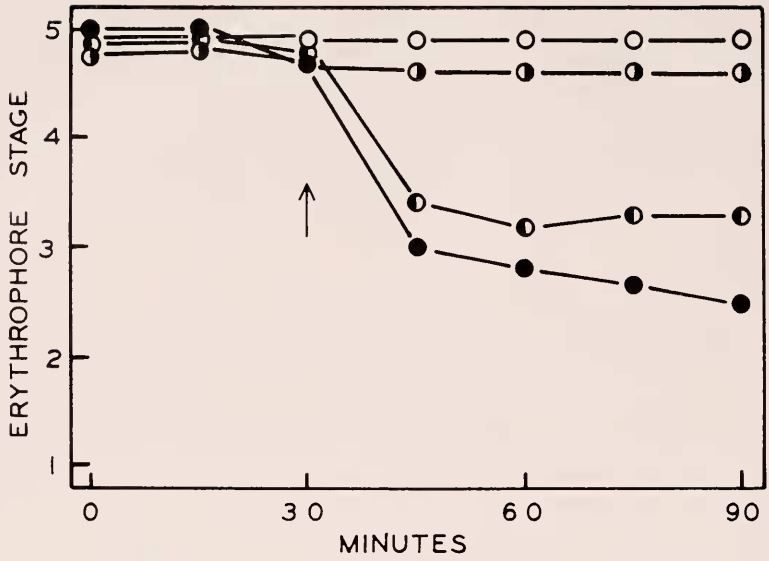

FIGURE 5. Relationships between the stages of erythrophores on the surface of the ovary and time in minutes. Circles represent erythrophores in saline alone; dots, erythrophores in saline from the outset and 30 minutes after 0 time tritocerebral commissure extract containing red pigment-concentrating hormone was added; circles half-filled on left, erythrophores in 1 mM colchicine from outset and 30 minutes after 0 time tritocerebral commissure extract was added; circles half-filled on right, erythrophores in 25 mM colchicine from outset and 30 minutes after 0 time tritocerebral commissure extract was added. The arrow shows when the hormone was added.

demonstrated that both colchicine and cytochalasin B inhibit the two processes of pigment dispersion and aggregation in any of the animal's chromatophores. As stated above, colchicine causes the disruption of microtubules; cytochalasin B, microfilaments. In amphibians colchicine inhibits melanin granule aggregation but not dispersion (Wright, 1955; Malawista, 1965, 1971a), whereas cytochalasin B strongly inhibits melanin dispersion but also has a less pronounced inhibitory effect on melanin granule aggregation (Malawista, 1971b; Magun, 1973). In the fiddler crab, *Uca pugilator,* colchicine inhibits only pigment aggregation while cytochalasin B clearly inhibits both aggregation and dispersion (Lambert and Crowe, 1973). In teleosts microtubules are required for both aggregation and dispersion of the pigment in melanophores (Murphy and Tilney, 1974). Interestingly, in contrast to the effects of cytochalasin B on other chromatophores that have been studied, the drug accelerated both dispersion and aggregation of pigment in the melanophores of the teleost *Oryzias latipes* (Ohta, 1974). In those species where colchicine and/or cytochalasin B inhibit pigment migration it has been previously postulated that microtubules and/or microfilaments respectively provide the propulsive force for movement of the pigment granules. Ohta, on the other hand, in light of his observation that cytochalasin B accelerates pigment migration in *Oryzias latipes,* suggested that in this fish microfilaments normally inhibit melanin granule movement.

Although the effects of colchicine and cytochalasin B on pigment migration have been attributed by previous investigators to disruption by these drugs of microtubules and microfilaments respectively, it is possible that these drugs could have other actions that might result in altering the rate of pigment granule migration. In the protozoan, *Tetrahymena pyriformis,* for example, colchicine impairs the mobility of membrane-intercalating particles, and this mobility does not appear to involve microtubules (Wunderlich, Müller, and Speth, 1973). Furthermore, both cytochalasin B and colchicine inhibited the (Na^+-K^+)-ATPase of rat liver plasma membranes (Bos and Emmelot, 1974). It is quite possible that the inhibition of pigment movement by 25 mM colchicine is not due to microtubule disruption because Robison and Charlton (1973) found that 1 mM colchicine disrupted erythrophore microtubules but had no appreciable effect on pigment aggregation. Recently, we have found (Lambert and Fingerman, manuscript in preparation) that 25 mM lumicolchicine, a derivative of colchicine which does not disrupt microtubules, is as effective as colchicine in inhibiting pigment aggregation in melanophores of the fiddler crab, *Uca pugilator,* which is further indication that the inhibitory action of the high concentrations of colchicine used herein may be at one or more sites other than microtubules. It is unlikely that colchicine is simply exerting a nonspecific poisoning action on these erythrophores because the effect of this drug is rapidly reversible. At this time, it cannot be definitely stated that cytochalasin B is active on microfilaments in erythrophores of *Palaemonetes vulgaris,* the plasma membrane being another possible site of action.

Fingerman (1969) presented a model for the actions of the red pigment-concentrating and pigment-dispersing hormone in *Palaemonetes* at the cellular level. These hormones were visualized as primarily altering the permeability of the cell membrane to the passage of ions, resulting in changes in the concentrations of sodium, potassium, and calcium in the erythrophore. The result of these ion concentration changes is activation of the mechanism that produces migration of the pigment in the appropriate direction that the hormone calls for, either centrifugal or centripetal. Furthermore, cyclic AMP is involved as the second messenger in the pigment-dispersing process (Fingerman, Hammond, and True, 1968). The present results enable us to extend this model one step further. The red-pigment-concentrating hormone through its effect on the potassium-sodium ratio in the cell may activate microfilaments which in turn would provide a propulsive force to the pigment-dispersing hormone through its effect on calcium permeability and/or its stimulation of the synthesis of its intermediary, cyclic AMP, would activate either the same or a different set of microfilaments to produce centrifugal migration of the pigment granules. A similar situation seems to hold for pigment dispersion in amphibian melanophores in which pigment dispersion induced by cyclic AMP is inhibited by cytochalasin B (Novales and Novales, 1972; McGuire, Moellmann, and McKeon, 1972; Magun, 1973; Fisher and Lyerla, 1974). How the propulsive force is actually applied to the granules has not been determined for any animal as yet.

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Summary

1. The effects of colchicine and cytochalasin B on pigment migration in integumentary erythrophores of the prawn, *Palaemonetes vulgaris,* were determined.

2. Colchicine in a concentration of 25 mM inhibited responses to both the red pigment-concentrating and red pigment-dispersing hormones by the integumentary erythrophores. Colchicine at a concentration of 5 mM did not inhibit the responses of these chromatophores to these hormones.

3. Cytochalasin B (10 μg/ml) also inhibited both centrifugal and centripetal migrations of the pigment granules in these integumentary erythrophores.

4. Colchicine in a 25 mM solution almost completely inhibited the response to red pigment-concentrating hormone in ovarian erythrophores whereas 1 mM colchicine produced only a slight inhibition of this response.

5. The integumentary erythrophores of *Palaemonetes vulgaris* are the first chromatophores that have been observed in any animal where both pigment dispersion and aggregation are inhibited by either colchicine or cytochalasin B.

6. These results are discussed in relation to previously published data from this and other laboratories.

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